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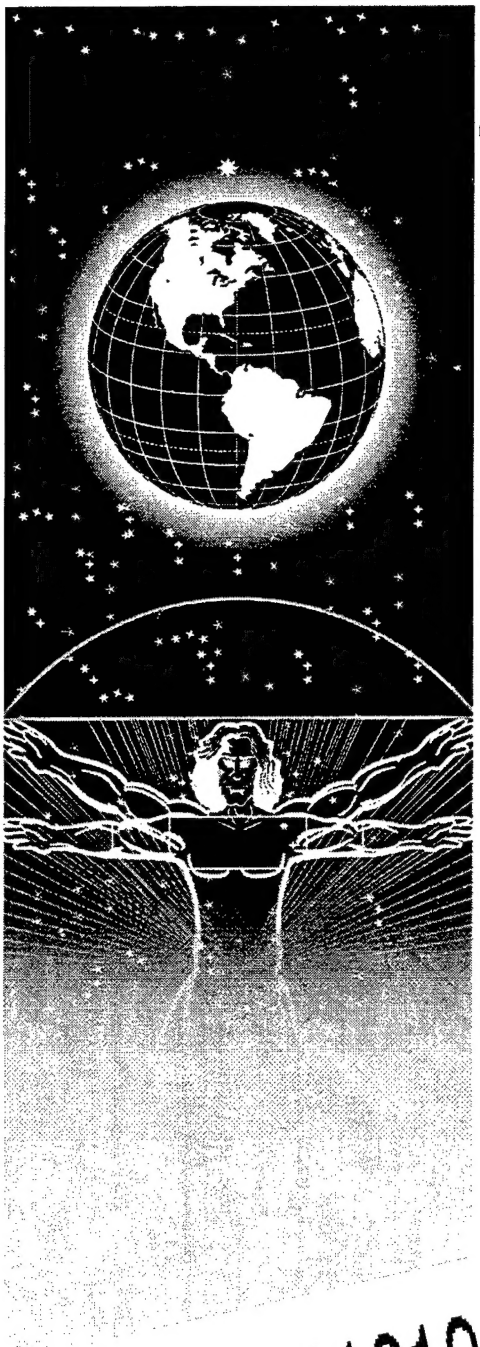

UNITED STATES AIR FORCE ARMSTRONG LABORATORY

Acute Toxicity Evaluation of JP-8 Jet Fuel and JP-8 Jet Fuel Containing Additives

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Terry A. Childress

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To reduce fuel fouling in current U.S Navy and Air Force aircraft systems and to provide additional heat sink and thermal stability for future systems, the Air Force is developing an improved JP-8 jet fuel (JP-8 + 100). Two companies (Betz and Mobil) have developed additive packages that are currently being tested in aircraft systems. To determine if the additive packages will produce health effects for flightline personnel, acute testing was performed on JP-8 and the two JP-8 + 100 jet fuels. A single oral dose at 5 mg jet fuel/kg body weight to five male and five female F-344 rats, and a single dermal application of 2 g jet fuel/kg body weight applied to five male and five female NZW rabbits resulted in no deaths. No signs of toxic stress were observed, and all animals gained weight over the 14-day observation periods. Single treatment of 0.5 mL neat jet fuel to rabbit skin produced negative results for skin irritation. Guinea pigs failed to elicit a sensitization response following repeated applications of the jet fuels. Inhalation vapor exposure to JP-8, JP-8 + 100 (Betz), and JP-8 (Mobil) were determined to be >3.43, >3.52, and >3.57 mg/L, respectively. LD_{50} values for aerosol exposure to JP-8, JP-8 + 100 (Betz), and JP-8 + 100 (Mobil) were >4.44, >4.39, and >4.54 mg/L, respectively. Under the conditions of these tests, the additive packages did not potentiate the acute effects normally associated with JP-8 jet fuel exposures.

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PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted at the Toxicology Division under the ManTech Geo-Centers Joint Venture Toxic Hazards Research Contract. This document serves as a final report on the in-life toxicity of JP-8 and JP-8 plus additives. The research described in this report began in November 1995 and was completed in February 1996 under Department of the Air Force Contract Nos. F33615-90-C-0532 and F41624-96-C-9010. Lt Col Terry A. Childress served as the Contracting Officer's Representative for the U.S. Air Force, Armstrong Laboratory. Darol E. Dodd, Ph.D., served as Program Manager for ManTech Geo-Centers Joint Venture. This study was sponsored by the U.S. Navy under the direction of LCDR Warren W. Jederberg, Naval Medical Research Institute/Toxicology Division.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals* prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institute of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

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ABBREVIATIONS

ALT	Alanine aminotransaminase
AST	Aspartate aminotransaminase
°C	Degrees Celcius
cfm	Cubic feet per minute
cm	Centimeter
°F	Degrees Fahrenheit
F-344	Fischer 344 (Rat)
ft	Foot
g	Gram
h	Hour
kg	Kilogram
L	Liter
LC_{50}	Median lethal concentration
LD_{50}	Median lethal dose
m^3	Meter cubed
mg	Milligram
min	Minute
mL	milliliter
mm	millimeter
NZW	New Zealand White (Rabbit)
P	Probability
SD	Standard deviation
SEM	Standard error of the mean
psi	Pounds per square inch
ppm	Parts per million
µm	micrometer

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SECTION 1

INTRODUCTION

The U.S. Navy and Air Force aircraft subsystems and engine heat loads are increasing rapidly. Fuel, within the aircraft, is used in thermal management systems to cool aircraft subsystems and the engine lubricating oil. The current thermal stresses are pushing JP-8 fuel to its thermal stability limits, resulting in fouling (coking) in engine fuel nozzles, afterburner spray assemblies, and manifolds. In some instances fuel degradation changes the spray pattern in the combustor or afterburner leading to damage to engine components. Certain aircraft are experiencing such severe problems with afterburner spraybar plugging that operational readiness is being adversely impacted. The Air Force has invested millions of dollars in advanced cleaning facilities to handle the resulting maintenance load. Some of these facilities generate hazardous waste, an additional cost factor.

To reduce fuel fouling in current systems and to provide additional heat sink and thermal stability for future systems, the U.S. Air Force is developing an improved JP-8 fuel (JP-8 + 100) that offers a 100 °F improvement in thermal stability and a 50% increase in fuel heat sink capability. Two companies (Betz and Mobil) have developed additive packages that are currently being tested in aircraft systems. Other companies (Exxon, DuPont, and Texaco) are in the process of developing additive packages. The JP-8 + 100 with the Betz package is being used currently in F-16s at Kingsley Air National Guard base and at Sheppard Air Force Base. The JP-8 + 100 with the Mobil package has been tested on a more limited basis.

In order to accurately evaluate the numerous additive packages, a number of physical, clinical, and health effect evaluations are required. Of particular concern to the Navy and Air Force industrial hygienists is whether the additive packages will potentiate health effects on flightline personnel. Previous acute testing of JP-8 fuel determined that it is nonirritating to eyes, slightly irritating to the skin, and has weak sensitizing potential

(Kinkead et al., 1992). Jet Fuel A, a commercial jet fuel similar to JP-8 but without additives, produced no dermal sensitization, minimal irritation to eyes, and a mild skin irritation potential (Vernot et al., 1990). Genetic toxicity tests revealed no mutagenicity and no evidence of genetic risk associated with JP-8 jet fuel (Brusick and Matheson, 1978a).

Long term toxicity testing of JP-8 was conducted by exposing F-344 rats and C57BL/6 mice to JP-8 vapors at 0, 500, and 1,000 mg/m^3 on a continuous basis for 90 days, followed by recovery until approximately 24 months of age. Evaluation of data revealed limited toxicity and no tumor formation. The toxicity seen was either not a direct treatment effect of JP-8 or was due to the male rat specific alpha 2-microglobulin protein droplet nephropathy. It is now recognized that the nephropathy seen in male rats is not expected to occur in humans. Therefore, the limited toxicity seen in the JP-8 repeated dose study was not relevant to humans (Mattie et al., 1991).

A developmental toxicity study of JP-8 indicated that JP-8 is not a teratogen in the rat (Cooper and Mattie, 1996). A reproductive toxicity study was also conducted for JP-8. Male rats were dosed with neat JP-8 (0, 750, 1500, 3000 mg/kg) daily by gavage for 90 days. Exposed male rats were mated to unexposed female rats after 70 days of exposure. The data on the male reproductive end points of this study have not been reported. Results of this study revealed a significant dose-dependent decrease in body weights of rats exposed to JP-8. The male rat-specific alpha 2-microglobin nephropathy was observed by histopathologic examination. A number of significant changes were also seen in blood and urine that were not dose-dependent. Additional treatment-related effects were a gastritis and a perianal dermatitis. Although there were no histopathological or weight changes in the livers of exposed rats, there was an increase in the liver enzymes AST and ALT. The elevated enzymes did not increase with increasing the dose of JP-8 (Mattie et al., 1995). Increases in liver enzymes, AST and ALT, have not been seen in previous subchronic jet fuel vapor studies (Brusick et al., 1978ab; Mattie et al., 1991; Gaworski et al., 1985; Kinkead et al., 1995). Parton et al. (1993) reported no increase in AST or ALT after

exposure of male rats to 500 or 1000 mg/m^3 aerosolized JP-8 for one hour daily for either 7 or 28 days. In addition, no liver pathology was reported after exposure to aerosolized JP-8 (Parton et al., 1993).

Female rats were dosed with neat JP-8 (0, 325, 750, 1500 mg/kg) daily by gavage for a total of 21 weeks (90-day oral study followed by gestation and lactation) in an effort to assess additional adverse effects which may be associated with prolonged exposure to this fuel. Results of this study revealed a significant dose-dependent decrease in body weights of the female rats. Significant organ weight ratio increases were also seen for the liver:body, liver:brain, and kidney:brain weights. Although liver enzymes were elevated in the male rat oral study, there was no increase in liver weight. Liver and spleen weights were also not different between control and male and female exposed rats after inhalation exposure to vapors of JP-8 for 90 days (Mattie et al., 1991). After inhalation of aerosolized JP-8, liver weights were not significantly higher than control rats, but relative liver weights were elevated in the high dose group (1000 mg/m^3) in both the 7 and 28 day repeated dose studies and in the low dose group (500 mg/m^3) in the 28-day study (Parton et al., 1993). There were no changes in urological, hematological, or clinical chemistry parameters. Corresponding histopathologic changes and increases in liver enzymes (ALT, AST) were not observed although there was an increase in liver weight. Significant pathological changes were limited to squamous hyperplasia of the stomach and perianal dermatitis (Mattie et al., 1996). The data on the female reproductive end points of this study have not been analyzed.

There are few studies of jet fuel toxicity. Many observed effects are attributed to the components of the fuel such as benzene, toluene, xylene, and n-hexane. Studies by Struwe et al. (1983) and Knave et al. (1976, 1978) have documented the occurrence of symptoms of neurasthenia, psychasthenia, and polyneuropathy in civilian and military aircraft workers. They reported cases of sexual dysfunction which were possibly neurologic in origin. Government reviews of the literature summarizing the health effects of jet fuel exposure have been published (Air Force, 1989; ATDR, 1993). The major

findings have been skin irritation and defatting, neurotoxicity, nephrotoxicity, and renal carcinogenicity in male rats. In a study by Brusick and Matheson (1978), significant preimplantation loss was observed after the fourth mating with male rats. Male mice showed significant testicular atrophy in a 12 month intermittent inhalation study (Bruner et al., 1993). Limited information is available on the reproductive effects of JP-4 and JP-8 jet fuels in humans and animals.

The approach for determining the acute toxicity of JP-8 + 100 was to perform a battery of acute tests as follows: oral, dermal, inhalation (vapor and aerosol), dermal irritation, and dermal sensitization. The acute toxicity battery was performed on the JP-8 + 100 with the Betz additive package, the JP-8 + 100 with the Mobil additive package, and on the JP-8 jet fuel as a comparison group. A JP-8 jet fuel group was necessary due to the variability that exists between fuels from different geographical locations and different refineries. The JP-8 alone group permitted a direct comparison of a sample of jet fuel currently in use with samples containing the new additives. In addition, it has been a number of years since JP-8 was originally tested for acute toxicity. Acute inhalation values, for both vapor and aerosol are not readily available in the literature as are irritation and sensitization data (Kinkead, et. al., 1992). A negative control group (no JP-8 treatment) was included in the testing battery for oral toxicity.

SECTION 2

MATERIALS

Test Agent

The JP-8 jet fuel and the two JP-8 + 100 fuel packages were supplied by the U.S. Air Force. In addition to the standard additives, the JP-8 + 100 (Betz) package contained Spec-Aid 8Q405 (100 ppm), DuPont Metal Deactivator (2 ppm), and BHT as an antioxidant (25 ppm); the JP-8 + (100) Mobil package contained MCP147B (150 ppm), DuPont Metal Deactivator (2 ppm), and BHT as an antioxidant (25 ppm). A sample of each test material will be maintained in the chemical archives located in Building 429.

Detailed chemical analysis of the JP-8 + 100 samples was not performed because it was done by other military groups as part of the fuel evaluation study. Figure 1 shows the gas chromatograms of the three test material as received. Figure 1 shows that the three samples appear to be identical.

Test Animals

Male and female Fischer-344 (F-344) rats weighing between 100 and 125, and 75 and 100 g, respectively, were purchased from Charles River Breeding Labs, Raleigh, NC. Male Hartley guinea pigs weighing approximately 300 g were purchased from Charles River Breeding Labs, Kingstown, NY. Male and female New Zealand white (NZW) rabbits weighing between 2 and 3 kg were purchased from Myrtle's Rabbitry Inc., Thompson's Station, TN. All animals were subjected to a two-week quarantine period. Rats were group housed (two per cage) in clear plastic cages with wood chip bedding. The guinea pigs and rabbits were housed individually; the guinea pigs in plastic cages with wood chip bedding, and the rabbits in wire-bottom, stainless-steel cages. Water and feed (Purina Rabbit Chow #5320, Purina Formulab #5008 for rats, and Purina Formulab #5025 for guinea pigs) were available *ad libitum*, except during the inhalation exposure period and for 16 h prior to oral dosing.

Animal room temperatures were maintained at 21 to 25 °C and the light/dark cycle was set at 12-h intervals.

SECTION 3

EXPERIMENTAL APPROACH

Skin Irritation

Six Male NZW rabbits per test material were clipped on the back and sides 24 h prior to dosing to allow for recovery of the skin from any abrasion resulting from the clipping. The test agents (0.5 mL) were applied to designated patch areas and were covered by a 3-cm square of surgical gauze two single layers thick. Strips of surgical adhesive tape held the gauze patch in place and the entire shaved area was covered with dental dam and secured with Vetrap® (3M Corp., Minneapolis, MN) and adhesive tape. The patches remained in place for 4 h; then all wrappings were removed and the residual test agent wiped from the skin. Test areas were evaluated for irritation using the Draize Table (Draize et al., 1959; Appendix A) as a reference standard at 4, 24, 48, and 72 h. Total scores of the four observations for all rabbits were divided by 24 to yield a primary irritation rating which was interpreted using the National Institute for Occupational Safety and Health skin test rating (Appendix B).

Sensitization

Prior to the start of the study, ten male guinea pigs per test material (30 animals total) were treated on the clipped left flank with 0.1 mL of the undiluted test material to determine the baseline irritation response. The site of the sensitization test was an area just behind the shoulder girdle. This site was clipped with an Oster® animal clipper and depilated with a commercial depilatory (Surgex Hair Remover Cream, Sparta Instrument Corp., Hayward, CA) 4 h prior to treatment. A Vetrap frame with a 1.5- x 1.5-cm opening was affixed to the guinea pig at the site of the depilated area. One-tenth of a mL of the test material was topically applied to the test area and covered with gauze, dental dam, and adhesive tape. This was done on Mondays, Wednesdays, and Fridays until a total of four sensitizing treatments were applied and evaluated. At the time of the third sensitizing treatment,

0.2 mL of a 25% aqueous dilution of TiterMax® adjuvant (Bacto Adjuvant Complete, Freund, Difco Laboratories, Detroit, MI) per animal was injected intradermally using two or three sites next to the test site. Following the fourth sensitizing treatment, the animals were rested for two weeks. Both flanks were then clipped and challenged on one flank with 0.1 mL of the test material. The challenge application was not occluded. The skin response at these sites was recorded at 4, 24, and 48 h after application (scoring method in Appendix C). Any animal eliciting a score of two or more at the test solution challenge site at the 48-h scoring interval was rated a positive responder. The percentage of animals responding was the important factor in determining sensitization potential. Appendix D was used to classify the test materials as to sensitization potential.

Oral Toxicity

Five male and five female F-344 rats per test material (15 male and 15 female total) were fasted 16 h prior to the administration of the oral dose. Each rat was weighed prior to dosing and 5 g/kg of neat compound was administered, via oral gavage. Surviving rats were weighed at 1, 2, 4, 7, 10, and 14 days postexposure. Signs of toxicity were recorded twice daily on symtomatology data sheets. On the 14th day postexposure, rats were sacrificed and gross pathology was performed for each animal.

Dermal Toxicity

Twenty-four hours prior to dosing, the back and sides of five male and five female NZW rabbits per test material (15 male and 15 female total) were clipped with an Oster® animal clipper. The undiluted dose of 2 g/kg was applied to the back of each rabbit and spread evenly to both sides. The dose was kept in place by applying an eight-ply gauze patch over the liquid. A clear plastic wrap was then applied over the entire midsection and was held in place with Vetrap® and elastoplast tape. The dose was kept in contact with the rabbit skin for 24 h. The tape, plastic wrap, and gauze were then removed and the residual test material was wiped from the animal. Animal body weights were recorded on days 1, 2, 4, 7, 10, and 14 posttreatment. Signs of toxicity and mortality were monitored and gross pathology was performed at the termination of the study.

Acute Inhalation Exposures

The limit test exposures were conducted in a stainless steel, 690-L Toxic Hazards Research Unit exposure chamber. The exposure chamber was operated in a dynamic (continuous flow) mode. The airflow through the chamber for the vapor exposures was maintained at a rate that provided 4 to 6 volumes of air per hour; the airflow for the vapor plus aerosol exposures provided 8 to 10 volumes of air per hour. The aerosol/vapor generation system consisted of two 250-mL round-bottom flasks each containing a six-jet Collison (BGI, Inc., Waltham, MA) compressed air nebulizer operated at a pressure of 62 pounds per square inch. The flask was kept in a 34 °C water bath. For the vapor exposures, an industrial HEPA filter (Model 007-0-08-2, Flanders Filters, Inc., Dallas, TX) was used to prevent aerosol from entering the chamber atmosphere. The flow produced was 2 cfm of saturated fuel vapor. For the vapor plus aerosol exposures, the filter was removed and an empty filter body used as an aging and elutriating chamber. An additional 2.4 cfm of air was added to aid in moving the vapor and aerosol through the system.

The jet fuel vapor exposure concentrations were analyzed using a Model 400 Hydrocarbon analyzer (Beckman Instrument Corp., Fullerton, CA) and quantified hexane vapor was used as a calibration standard. The vapor-only

exposures were monitored for presence of aerosol with a Ram-S aerosol mass analyzer (GCA Corp., Bedford, MA) operated in the 200 µg/L mode. No aerosol was observed by Ram-S or by weighing the hydrocarbon analyzer filters.

The jet fuel aerosol concentrations were monitored by using a filter in-line before the hydrocarbon analyzer (Extra Thick Glass Fiber Filter, 25 mm, Gelman Sciences, Ind., Ann Arbor, MI). An airflow of 3 L/min was maintained to the hydrocarbon analyzer. The filtered vapor sample was analyzed using the hydrocarbon analyzer, and three 20 second filter samples were taken for mass analysis of the aerosol. The hydrocarbon analyzer filters were replaced every 15 minutes to maintain the 3 L/min air flow. The aerosol concentration exceeded the Ram-S maximum analytical level.

Five male and five female F-344 rats per test material (15 male and 15 female total) were placed in the 690-L chamber and exposed for 4 h to a target 5 mg/L (Limit Test) concentration of vaporized and aerosolized test material. Records were maintained for body weights (Day 0, 7, 10, and 14 postexposure), signs of toxicity, and mortality. At sacrifice, gross pathology was performed and lungs were removed for histopathologic evaluation.

Statistical Analysis

Mean body weights and body weight gains of the inhalation rats were compared using a three factorial repeated measures analysis of variance (Johnson and Wichern, 1988). The factors were treatment, sex, and type of exposure (vapor or aerosol). The repeated measure was the difference between body weights which were measured on Days 0, 1, 2, 7, 10, and 14. If the overall F was significant ($p < 0.05$), Bonferroni multiple comparisons were done to find pairwise comparisons (Johnson and Wichern, 1988). A probability of 0.05 or less inferred a significant change between groups.

SECTION 4

RESULTS

Skin Irritation

Six rabbits per test material were treated dermally with 0.5 mL of either JP-8, JP-8 + 100 (Mobil), or JP-8 + 100 (Betz). Slight erythema was noted in two of the six JP-8-treated animals immediately following 4-h dermal contact with the test material. No erythema, edema, or necrosis was observed in any of the JP-8 + 100 (Betz)- or the JP-8 + 100 (Mobil)-treated rabbits upon examination following 4-h dermal contact (Table 1). Subsequent irritation observations at 24, 48, and 72 h were all negative for the JP-8 + 100 (Betz)-treated animals. Three JP-8-treated animals displayed mild erythema from
24-h through 72-h posttreatment, and three JP-8 + 100 (Mobil) animals displayed mild erythema beginning at 48-h posttreatment. Primary skin indices for JP-8, JP-8 + 100 (Mobil), and JP-8 + 100 (Betz) were determined to be 0.5, 0.25, and 0.0, respectively.

Sensitization

No test animals exhibited edema following the baseline response treatment of 0.1 mL test material to the shaved flank. All three test materials caused very slight to slight erythema by the 24-h posttreatment observations (4/10, 5/10, and 3/10 for JP-8, JP-8 + 100 (Betz), and JP-8 + 100 (Mobil), respectively). Very slight to slight erythema continued through the 48-h observations (4/10, 6/10, and 4/10 for JP-8, JP-8 + 100 (Betz), and JP-8 + 100 (Mobil), respectively). Following 10 days of sensitization dosing and two weeks of rest, the test animals were challenged with 0.1 mL of the test material. All three test materials produced no edema at 24 and 48 h after the challenge treatment. All three test materials produced very slight to well-defined erythema (Table 2). There were no differences between sensitizing and challenge dose effects.

Oral Toxicity

Five male and five female rats per test material were orally dosed at 5 g/kg body weight with either JP-8, JP-8 + 100 (Mobil), or JP-8 + 100 (Betz). No deaths resulted from the oral administration of the test agents. Clinical observations noted after oral gavage treatment included lethargy and shallow breathing for all fuel-treated animals. By 24-h posttreatment, all animals appeared normal. All rats gained weight during the 14-day posttreatment observation period (Tables 3 & 4); however, all fuel-treated male rats and the JP-8 + 100 (Mobil)-treated female rats had slight depression (but not statistically significant) in body weight gains compared to controls.

Dermal Toxicity

Five male and five female rabbits per test material were treated at 2 g/kg body weight with either JP-8, JP-8 + 100 (Mobil), or JP-8 + 100 (Betz). No mortality occurred due to treatment with the test agents. Clinical signs noted during the 14-day observation period included mild erythema and coriaceous skin at the site of test material application. These observations were noted for animals of all three test groups. One female rabbit from the JP-8 treatment group died of accidental injury immediately after being dermally treated. All rabbits in the JP-8 and JP-8 + 100 (Mobil) gained weight during the 14-day observation period (Tables 5 & 6). Seven out of 10 JP-8 + 100 (Betz) animals gained weight over the observation period; two animals maintained their initial weights, and one animal lost weight during the observation period.

Table 1. Primary Skin Irritation Scores Following Dermal Contact with JP-8, JP-8 + 100 (Mobil), and JP-8 + 100 (Betz)

JP-8

Rabbit No.	Examination Time (Hours Posttreatment) 4	24	48	72
13	1	2	0	0
14	0	0	0	0
15	1	0	0	0
16	0	1	1	2
17	0	0	0	1
18	0	0	0	0

JP-8 + 100 (Mobil)

Rabbit No.	Examination Time (Hours Posttreatment) 4	24	48	72
01	0	0	0	0
02	0	0	1	0
03	0	0	0	0
04	0	0	1	2
05	0	0	1	1
06	0	0	0	0

JP-8 + 100 (Betz)

Rabbit No.	Examination Time (Hours Posttreatment) 4	24	48	72
07	0	0	0	0
08	0	0	0	0
09	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0

Table 2. Skin Sensitization Test Scores for Challenge Application of JP-8, JP-8 + 100 (Mobil), and JP-8 + 100 (Betz)

Guinea Pig No.	Erythema/Edema (Hours Posttreatment) 4	24	48
		JP-8	
10	1/0	1/0	1/0
11	1/0	1/0	0/0
14	1/0	1/0	0/0
15	1/0	1/0	0/0
16	1/0	1/0	0/0
17	1/0	1/0	0/0
18	1/0	0/0	1/0
19	1/0	1/0	1/0
20	1/0	1/0	0/0
21	1/0	1/0	1/0
		JP-8 + 100 (Mobil)	
34	1/0	2/0	1/0
35	1/0	1/0	1/0
36	1/0	0/0	0/0
39	1/0	1/0	0/0
40	1/0	1/0	0/0
41	1/0	1/0	1/0
42	1/0	0/0	0/0
43	1/0	1/0	0/0
44	1/0	1/0	0/0
45	1/0	1/0	1/0
		JP-8 + 100 (Betz)	
22	1/0	1/0	1/0
23	1/0	1/0	1/0
24	0/0	0/0	0/0
25	1/0	1/0	0/0
26	1/0	1/0	1/0
28	1/0	1/0	1/0
29	1/0	0/0	0/0
30	1/0	1/0	0/0
31	1/0	0/0	0/0
33	1/0	1/0	0/0

Positive Responders = 0%
Classification = Non-Responder

Table 3. Body Weights (g) of Male Rats After Oral Gavage with 5 g/kg JP-8, JP-8 + 100 (Mobil), and JP-8 + 100 (Betz)

	Days Posttreatment						
Rat No.	0	1	2	4	7	10	14
				Control[a]			
1	132.4	147.0	149.7	157.4	175.1	188.4	207.2
2	134.5	150.6	153.5	160.1	177.7	189.7	206.3
3	130.7	147.8	151.3	159.6	177.5	191.0	208.9
4	130.9	150.9	154.0	161.4	183.8	198.4	214.1
5	137.4	155.6	161.0	169.3	188.3	204.5	222.2
Mean	**133.2**	**150.4**	**153.9**	**161.6**	**180.5**	**194.4**	**211.7**
(± S.E.M)	**(2.8)**	**(3.4)**	**(4.3)**	**(4.6)**	**(5.4)**	**(6.8)**	**(6.6)**
				JP-8			
6	122.8	118.0	125.4	131.2	149.0	162.7	171.9
7	126.1	132.2	143.1	146.9	164.0	179.8	188.5
8	133.3	133.8	144.6	153.0	174.6	187.6	200.1
9	133.2	130.9	140.3	151.6	175.5	190.6	208.3
10	120.3	123.0	132.5	138.3	157.3	170.5	182.8
Mean	**127.1**	**127.6**	**137.2**	**144.2**	**164.1**	**178.2**	**190.3**
(± S.E.M)	**(5.9)**	**(6.8)**	**(8.1)**	**(9.3)**	**(11.3)**	**(11.7)**	**(14.3)**
				JP-8 + 100 (Mobil)			
11	128.0	136.6	141.2	147.9	163.0	176.4	191.7
12	130.3	137.5	142.1	147.8	164.6	176.6	192.2
13	130.5	138.6	142.3	148.4	164.8	177.5	191.8
14	131.2	141.0	143.6	149.5	169.8	183.1	203.8
15	128.1	122.7	129.7	138.8	159.2	172.5	188.6
Mean	**129.6**	**135.3**	**139.8**	**146.5**	**164.3**	**177.2**	**193.6**
(± S.E.M)	**(1.5)**	**(7.2)**	**(5.7)**	**(4.3)**	**(3.8)**	**(3.8)**	**(5.9)**
				JP-8 + 100 (Betz)			
16	141.6	150.5	161.9	164.7	187.6	201.1	218.8
17	139.0	149.8	154.8	161.1	180.9	191.8	208.4
18	121.1	121.9	129.7	135.6	152.9	163.9	172.2
19	132.9	138.4	149.1	155.9	176.0	190.4	205.3
20	130.3	123.6	135.1	140.2	162.9	175.4	187.1
Mean	**133.0**	**136.8**	**146.1**	**151.5**	**172.1**	**184.5**	**198.4**
(± S.E.M)	**(8.0)**	**(13.7)**	**(13.5)**	**(12.9)**	**(14.0)**	**(14.8)**	**(18.6)**

[a]Control animals received 1 mL sterile H_20/100g body weight.

Table 4. Body Weights of Female Rats After Oral Gavage with 5 g/kg JP-8, JP-8 + 100 (Mobil), and JP-8 + 100 (Betz)

Rat No.	Days Posttreatment 0	1	2	4	7	10	14
				Control[a]			
31	98.6	113.4	113.4	117.4	127.5	135.4	141.7
32	90.9	102.9	104.1	107.8	119.7	127.8	132.0
33	91.6	105.0	107.7	108.8	121.1	126.1	132.3
34	95.1	107.1	110.9	111.3	121.0	127.7	131.9
35	99.6	110.9	115.2	117.1	128.2	132.2	139.6
Mean	**95.2**	**107.9**	**110.3**	**112.5**	**123.5**	**129.8**	**135.5**
(± S.E.M)	**(3.9)**	**(4.3)**	**(4.4)**	**(4.5)**	**(4.0)**	**(3.8) (4.8)**	
				JP-8			
36	101.5	103.2	109.2	110.6	124.4	128.8	138.4
37	95.4	99.1	104.1	105.9	120.2	126.7	129.2
38	101.6	106.6	113.0	116.6	130.4	134.3	138.6
39	101.8	109.0	113.3	115.3	127.1	134.9	140.9
40	94.8	99.6	104.2	107.7	121.4	126.5	135.6
Mean	**99.0**	**103.5**	**108.8**	**111.2**	**124.7**	**130.2**	**136.5**
(± S.E.M)	**(3.6)**	**(4.3)**	**(4.5)**	**(4.7)**	**(4.2)**	**(4.1)**	**(4.5)**
				JP-8 + 100 (Mobil)			
41	94.6	97.5	104.5	107.4	118.6	125.4	130.4
42	98.1	103.3	109.0	111.0	122.0	128.6	135.9
43	96.2	100.4	104.4	105.4	117.6	124.7	133.4
44	97.3	103.5	106.4	107.7	120.4	126.8	133.9
45	100.8	99.4	106.8	103.7	117.6	125.3	132.5
Mean	**97.4**	**100.8**	**106.2**	**107.0**	**119.2**	**126.2**	**133.2**
(± S.E.M)	**(2.3)**	**(2.6)**	**(1.9)**	**(2.7)**	**(1.9)**	**(1.6)**	**(2.0)**
				JP-8 + 100 (Betz)			
46	99.7	105.3	110.1	113.5	123.4	127.7	134.9
47	99.6	104.5	110.7	113.2	126.0	133.0	142.0
48	93.6	97.8	103.6	105.3	118.1	127.3	133.6
49	98.9	101.0	105.6	110.8	122.6	131.8	136.7
50	101.6	101.6	106.8	110.5	125.7	129.7	138.3
Mean	**98.7**	**102.0**	**107.4**	**110.7**	**123.2**	**129.9**	**137.1**
(± S.E.M)	**(3.0)**	**(3.0)**	**(3.0)**	**(3.3)**	**(3.2)**	**(2.5)**	**(3.3)**

[a]Control animals received 1 mL sterile H_20/100g body weight.

Table 5. Body Weights (kg) of Male Rabbits After 24-Hour Dermal Exposure to 2 g/kg JP-8, JP-8 + 100 (Mobil), and JP-8 + 100 (Betz)

	Days Posttreatment					
Rabbit No.	0	1	2	4	10	14
			JP-8			
19	2.7	2.5	2.6	2.7	2.6	3.0
20	2.8	2.6	2.7	2.8	2.9	3.0
21	2.9	2.7	2.9	3.0	3.1	3.2
22	2.8	2.7	2.8	2.8	2.8	3.1
23	2.5	2.4	2.4	2.6	2.8	2.9
Mean	**2.7**	**2.6**	**2.7**	**2.8**	**2.8**	**3.0**
(± S.E.M)	**(0.2)**	**(0.1)**	**(0.2)**	**(0.1)**	**(0.2)**	**(0.1)**
			JP-8 + 100 (Mobil)			
29	2.9	2.8	2.9	3.0	3.1	3.2
30	3.2	3.0	3.0	3.2	3.7	3.5
31	3.0	2.7	2.9	3.0	3.2	3.1
32	2.9	2.7	2.9	2.9	3.1	3.0
33	2.7	2.6	2.6	2.5	2.7	2.8
Mean	**2.9**	**2.8**	**2.9**	**2.9**	**3.2**	**3.1**
(± S.E.M)	**(0.2)**	**(0.2)**	**(0.2)**	**(0.3)**	**(3.4)**	**(0.3)**
			JP-8 + 100 (Betz)			
24	2.7	2.6	2.7	2.8	2.6	2.9
25	2.9	2.6	2.8	2.9	3.0	3.1
26	2.6	2.5	2.6	2.8	2.6	2.6
27	3.0	2.7	2.9	3.0	3.0	3.3
28	3.2	2.9	3.0	3.2	3.1	2.9
Mean	**2.9**	**2.7**	**2.8**	**2.9**	**2.9**	**3.0**
(± S.E.M)	**(0.2)**	**(0.2)**	**(0.2)**	**(0.2)**	**(0.2)**	**(0.3)**

Table 6. Body Weights (kg) of Female Rabbits After 24-Hour Dermal Exposure to 2 g/kg JP-8, JP-8 + 100 (Mobil), and JP-8 + 100 (Betz)

	Days Posttreatment					
Rabbit No.	0	1	2	4	10	14
			JP-8			
34	2.5	[a] --	--	--	--	--
35	2.8	2.6	2.8	2.8	3.1	3.2
36	2.7	2.5	2.6	2.6	2.8	2.8
37	2.6	2.4	2.5	2.8	2.7	2.7
38	3.0	2.8	2.9	3.0	3.2	3.4
Mean	**2.7**	**2.6**	**2.7**	**2.8**	**3.0**	**3.0**
(± S.E.M)	**(0.2)**	**(0.2)**	**(0.2)**	**(0.2)**	**(0.2)**	**(0.3)**
			JP-8 + 100 (Mobil)			
44	2.8	2.6	2.7	2.8	3.0	3.0
45	2.9	2.7	2.9	2.9	3.2	3.2
46	2.5	2.4	2.5	2.6	2.8	2.7
47	3.0	2.8	2.9	2.9	3.2	3.2
48	2.8	2.6	2.7	2.8	3.1	3.0
Mean	**2.8**	**2.6**	**2.7**	**2.8**	**3.1**	**3.0**
(± S.E.M)	**(0.2)**	**(0.1)**	**(0.2)**	**(0.1)**	**(0.2)**	**(0.2)**
			JP-8 + 100 (Betz)			
39	2.9	2.7	2.8	2.9	2.8	3.2
40	2.9	2.7	2.8	2.9	2.9	3.1
41	2.6	2.4	2.5	2.6	2.8	3.3
42	2.5	2.5	2.5	2.6	2.6	2.6
43	2.6	2.4	2.6	2.7	2.7	2.9
Mean	**2.7**	**2.5**	**2.6**	**2.7**	**2.8**	**3.0**
(± S.E.M)	**(0.2)**	**(0.2)**	**(0.2)**	**(0.2)**	**(0.1)**	**(0.3)**

[a]Animal died from self inflicted injury.

Inhalation Toxicity

Vapor Exposures

The limit test concentration of 5 mg/L was not obtainable in the vapor only exposures. The system limit of when a condensate aerosol was detected with the Ram-S was between 3.7 and 4.0 mg/L, so the maximum concentration for vapor only exposures was 3.7 mg/L.

No aerosol was detected during the vapor-only exposures. Chamber air flow was limited to 2 cfm of saturated vapor. Table 7 contains the vapor exposure concentration data summary.

All rats survived 4-h inhalation exposure to vapors of JP-8, JP-8 + 100 (Mobil), and JP-8 + 100 (Betz). During exposure, test animals exposed to JP-8 and JP-8 + 100 (Mobil) vapors demonstrated signs of eye or upper respiratory irritation. All treated animals gained weight during the 14-day observation period (Tables 8 and 9). Combined weight differences from study Days 0 to 1 for the JP-8 animals were statistically significantly lower ($p < 0.01$) than both the JP-8 + 100 (Betz) and JP-8 + 100 (Mobil) groups (Figure 2). Weight gains from Day 2 to Day 7, and from Day 10 to Day 14 for the JP-8 and JP-8 + 100 (Mobil) groups differed ($p < 0.01$) from the weights of the JP-8 + 100 (Betz) group. Gross observations at sacrifice failed to reveal any treatment-related lesions.

Aerosol Exposures

The vapor plus aerosol exposures were performed using the same generation system as the vapor only exposures, except the HEPA filter was removed to allow the aerosol to pass through into the exposure chamber. Additional air was supplied to move the vapor and aerosol through the system. The total air flow for the vapor and aerosol exposures averaged 3.5 cfm. One impactor sample (30 seconds at 20 L/min flow) was taken approximately 2 h into each exposure. The ratio of the mass of vapor to the mass of aerosol was approximately 3:2 during exposure. Table 7 contains the vapor plus aerosol exposure concentration data summary.

All rats survived 4-h inhalation exposure to vapor and aerosol of JP-8, JP-8 + 100 (Mobil), and JP-8 + 100 (Betz). All treated animals gained weight over the 14-day observation period (Tables 10 and 11). Statistical analyses of the difference between group body weights over the 14-day observation period determined there were no differences among the treatment levels (Figure 3). Gross observations at sacrifice failed to reveal any treatment-related lesions.

Appendix E shows data points and plots from chromatograms taken of JP-8 + 100 (Mobil) for the material as received, the material in vapor phase, the aerosol generated, and the spent material from the aerosol exposure. Chromatography from JP-8 and JP-8 + 100 (Betz) are indistinguishable (see Figure 1) from that of JP-8 + 100 (Mobil), so therefore, the plots and data for these jet fuels should be similar.

Figure 1. Gas Chromatograms of Liquid JP-8, JP-8 + 100 (Mobil), and JP-8 + 100 (Betz) Samples[a] as Received

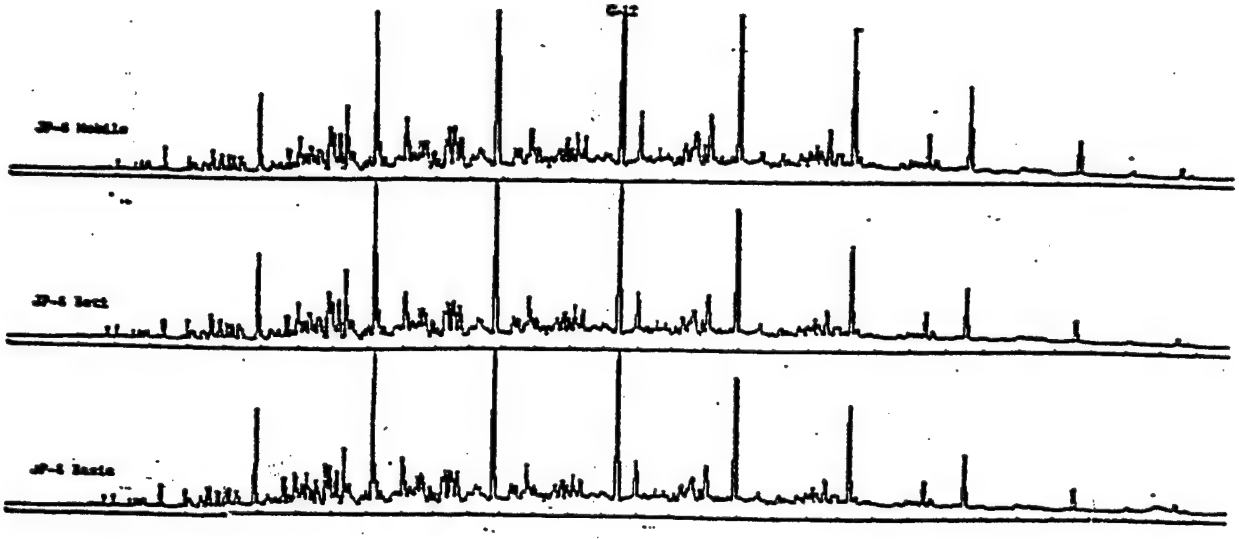


Table 7. Data Summary for the Limit Test Inhalation Exposures of JP-8, JP-8 + 100 (Betz), and JP-8 + 100 (Mobil)

Test Material	Statistic Mass	Vapor mG/L	Aerosol mG/L	Total mG/L	Aerosol Statistics	Microns
		VAPOR				
JP-8	MEAN	3.43	<0.0001	3.43		
	STDEV	0.29		0.29		
JP-8 + 100 Betz	MEAN	3.52	<0.0001	3.52		
	STDEV	0.16		0.16		
JP-8 + 100 Mobil	MEAN	3.57	<0.0001	3.57		
	STDEV	0.18		0.18		
		VAPOR + AEROSOL				
JP-8	MEAN	2.63	1.81	4.44	MMD	1.79
	STDEV	0.34	0.20	0.562	SIGMA-G	1.60
JP-8 + 100 Betz	MEAN	2.52	1.87	4.39	MMD	1.89
	STDEV	0.34	0.53	0.73	SIGMA-G	1.54
JP-8 + 100 Mobil	MEAN	2.61	1.93	4.54	MMD	1.86
	STDEV	0.30	0.18	0.47	SIGMA-G	1.50

Table 8. Body Weights (g) of Male Rats After 4-Hour Inhalation Exposure to Vapors of JP-8, JP-8 + 100 (Mobil),and JP-8 + 100 (Betz)

	Days Posttreatment					
Rat No.	0	1	2	7	10	14
			JP-8			
21	198.2	195.4	197.2	213.3	221.5	224.6
22	179.0	174.4	176.7	190.6	197.4	198.4
21	198.7	191.0	192.8	202.8	205.5	207.3
22	181.0	178.2	183.1	192.8	194.8	198.5
23	201.7	198.8	200.7	217.9	223.6	232.7
Mean	**191.7**	**187.6**	**190.1**	**203.5**	**208.6**	**212.3**
(± S.E.M)	**(4.8)**	**(4.8)**	**(4.5)**	**(5.4)**	**(6.0)**	**(7.0)**
			JP-8 + 100 (Mobil)			
61	215.9	220.8	223.4	242.5	251.3	261.8
62	215.6	215.5	218.8	233.5	239.9	251.1
63	221.1	217.8	220.7	235.3	244.0	254.5
64	221.8	222.2	225.3	242.1	250.9	259.3
65	217.9	215.6	220.8	236.4	242.6	254.3
Mean	**218.5**	**218.4**	**221.8**	**238.0**	**245.7**	**256.2**
(± S.E.M)	**(1.3)**	**(1.4)**	**(1.1)**	**(1.8)**	**(2.3)**	**(1.9)**
			JP-8 + 100 (Betz)			
26	214.1	213.2	213.8	224.3	230.1	254.2
27	223.9	222.4	224.8	239.3	246.0	263.4
28	198.7	198.9	200.3	205.4	210.6	222.1
29	200.9	202.3	203.5	212.6	218.5	235.7
30	206.4	206.4	207.6	216.9	222.6	237.9
Mean	**208.8**	**208.6**	**210.0**	**219.7**	**225.6**	**242.7**
(± S.E.M)	**(4.6)**	**(4.2)**	**(4.3)**	**(5.8)**	**(6.0)**	**(7.2)**

Table 9. Body Weights (G) of Female Rats After 4-Hour Inhalation Exposure to Vapors of JP-8, JP-8 + 100 (Mobil), and JP-8 + 100 (Betz)

	Days Posttreatment					
Rat No.	0	1	2	7	10	14
			JP-8			
51	127.4	122.8	125.1	130.1	133.5	131.7
52	127.8	123.0	124.7	130.2	132.3	130.8
53	131.6	125.0	125.4	129.2	131.6	132.9
54	138.8	131.2	134.0	139.8	144.2	142.6
55	133.5	128.5	130.2	134.4	131.3	134.4
Mean	**131.8**	**126.1**	**127.9**	**132.7**	**134.6**	**134.5**
(± S.E.M)	**(2.1)**	**(1.6)**	**(1.8)**	**(2.0)**	**(2.4)**	**(2.1)**
			JP-8 + 100 (Mobil)			
81	154.3	155.7	156.6	159.7	161.2	160.0
82	150.6	152.4	154.1	157.5	160.9	162.8
83	146.7	148.1	151.4	156.4	153.9	155.8
84	136.8	138.8	141.4	147.9	150.6	154.8
85	142.2	142.1	144.9	151.8	154.4	155.5
Mean	**146.1**	**147.4**	**150.0**	**154.7**	**156.2**	**157.8**
(± S.E.M)	**(3.1)**	**(3.1)**	**(2.8)**	**(2.1)**	**(2.1)**	**(1.6)**
			JP-8 + 100 (Betz)			
56	132.7	133.0	134.7	132.5	133.9	148.9
57	143.4	141.4	146.1	145.5	150.9	158.6
58	142.1	143.6	143.9	143.8	150.0	157.5
59	138.1	134.2	138.1	137.3	141.9	151.0
60	121.6	120.5	123.9	126.3	125.4	139.6
Mean	**135.6**	**134.5**	**137.3**	**137.1**	**140.4**	**151.1**
(± S.E.M)	**(4.0)**	**(4.1)**	**(3.9)**	**(3.6)**	**(4.9)**	**(3.4)**

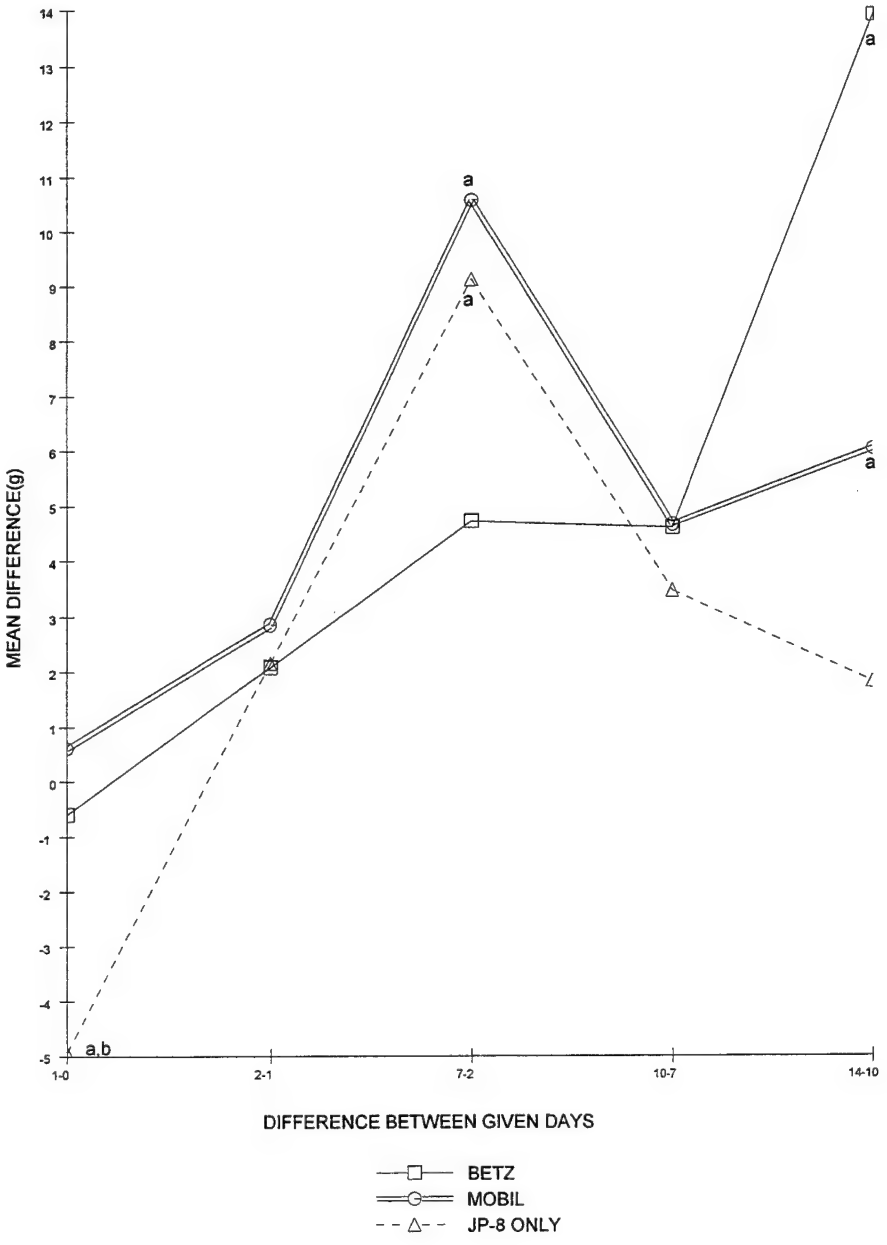
Table 10. Body Weights (g) of Male Rats After 4-Hour Inhalation Exposure to Vapors Plus Aerosols of JP-8,JP-8 + 100 (Mobil), and JP-8 + 100 (Betz)

Rat No.	Days Posttreatment 0	1	2	7	10	14
			JP-8			
67	289.2	280.5	286.1	295.9	301.6	308.6
68	279.6	271.8	276.4	285.0	288.9	297.8
69	285.7	275.9	278.1	289.8	297.4	304.0
70	275.7	256.7	262.4	277.1	282.0	289.7
71	283.3	274.1	278.5	292.1	298.6	307.1
Mean	**282.7**	**271.8**	**276.3**	**288.0**	**293.7**	**301.4**
(± S.E.M)	**(2.3)**	**(4.0)**	**(3.9)**	**(3.2)**	**(3.6)**	**(3.5)**
			JP-8 + 100 (Mobil)			
76	284.3	277.5	276.3	288.2	293.8	302.2
77	258.4	255.1	257.7	264.4	268.2	276.0
78	280.6	273.5	277.6	290.7	292.2	302.9
79	291.0	273.5	286.3	299.8	302.7	313.3
80	293.6	285.1	285.4	298.4	302.9	312.0
Mean	**281.6**	**272.9**	**276.7**	**288.3**	**292.0**	**301.3**
(± S.E.M)	**(6.2)**	**(4.9)**	**(5.1)**	**(6.4)**	**(6.3)**	**(6.7)**
			JP-8 + 100 (Betz)			
66	279.2	272.7	273.5	281.9	287.9	295.6
72	300.8	288.4	296.9	303.0	309.2	317.8
73	259.2	244.2	249.5	255.9	264.2	274.7
74	281.0	272.4	276.1	284.3	290.4	299.5
75	302.8	296.1	300.8	309.3	314.8	331.4
Mean	**284.6**	**274.8**	**279.4**	**286.9**	**293.3**	**303.8**
(± S.E.M)	**(8.0)**	**(8.9)**	**(9.2)**	**(9.4)**	**(8.9)**	**(9.7)**

Table 11. Body Weights (g) of Female Rats After 4-Hour Inhalation Exposure to Vapors Plus Aerosols of JP-8, JP-8 + 100 (Mobil), and JP-8 + 100 (Betz)

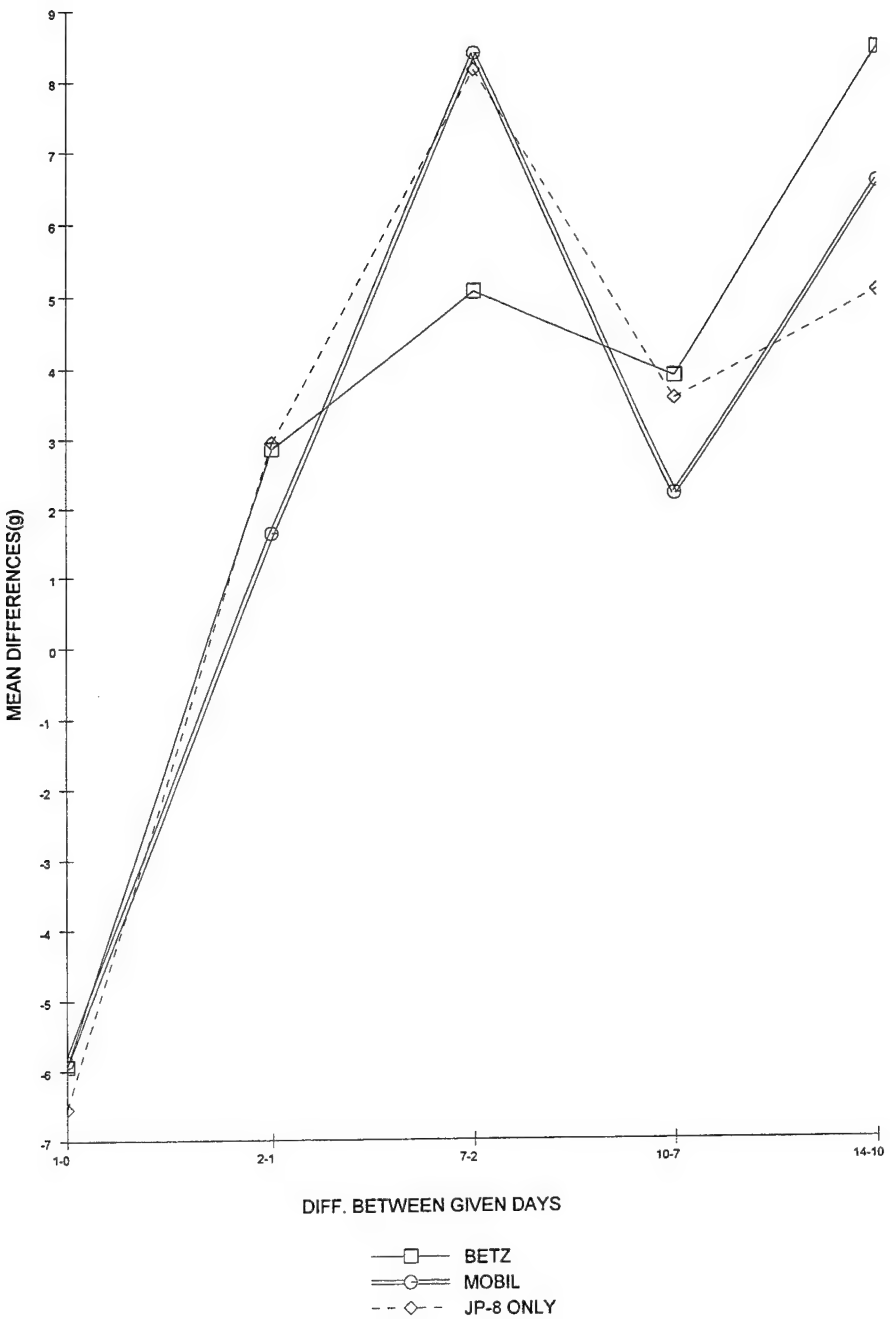
	Days Posttreatment					
Rat No.	0	1	2	7	10	14
			JP-8			
86	158.1	156.4	159.0	162.7	166.3	161.9
87	158.9	154.6	156.1	161.0	160.2	162.9
88	163.5	161.8	162.8	165.0	165.4	169.7
89	170.1	166.7	168.9	173.1	176.0	178.5
90	156.9	157.0	156.6	164.6	165.3	171.7
Mean	**161.5**	**159.3**	**160.7**	**165.3**	**166.6**	**168.9**
(± S.E.M)	**(2.4)**	**(2.2)**	**(2.4)**	**(2.1)**	**(2.6)**	**(3.0)**
			JP-8 + 100 (Mobil)			
96	170.1	166.0	167.3	174.8	173.7	179.0
97	158.4	155.7	154.9	159.2	160.4	163.4
98	163.8	160.2	159.5	163.1	166.1	167.7
99	174.5	173.1	171.6	175.5	176.0	182.1
100	166.0	162.4	161.8	168.0	167.9	170.6
Mean	**166.6**	**163.5**	**163.0**	**168.1**	**168.8**	**172.6**
(± S.E.M)	**(2.7)**	**(2.9)**	**(2.9)**	**(3.2)**	**(2.8)**	**(3.5)**
			JP-8 + 100 (Betz)			
91	164.3	158.3	158.2	162.2	164.2	170.7
92	161.8	160.8	159.7	162.6	164.5	170.5
93	160.5	160.4	162.3	164.7	164.2	169.2
94	154.3	152.5	156.1	157.0	158.3	164.7
95	145.0	143.7	144.8	147.4	149.2	156.9
Mean	**157.2**	**155.1**	**156.2**	**158.8**	**160.1**	**166.4**
(± S.E.M)	**(3.5)**	**(3.2)**	**(3.0)**	**(3.1)**	**(3.0)**	**(2.6)**

Figure 2. Combined Body Weights of Male and Female Rats After Exposure to Vapors of JP-8, JP-8 +100 (Mobil) or JP-8 + 100 (Betz)



a = significantly different than Betz at $p < 0.01$.
b = significantly different than Mobil at $p < 0.01$.

Figure 3. Combined Body Weights of Male and Female Rats After Exposure to Aerosols of JP-8,JP-8 + 100 (Mobil) or JP-8 + 100 (Betz)



SECTION 5

DISCUSSION

In the oral and dermal toxicity studies, no deaths or toxic signs were observed in any of the animals, and body weight gains during the subsequent 14-day observation period appeared to be unaffected by treatment. Remarkable irritating effects were not observed as a result of exposure to intact skin of rabbits. The results of animal irritation studies do not predict irritation of human skin reported by military personnel (Bell et al., 1996). Acute inhalation of the vapors or aerosols of the test materials near limit concentrations produced no mortality in male and female F-344 rats. The acute toxicity results reported in this report for the JP-8 and JP-8 + 100 jet fuels are similar to those reported for Jet Fuel A (Vernot et al., 1990). The results differ slightly from those in Kinkead et al. (1992) which reported JP-8 fet fuel had a weak sensitizing potential. No sensitization reaction in guinea pigs occured in this study. Again the sensitization results in animal studies are not a good predictor of sensitization of human skin reported by military personnel (Gould, 1996). Differences between the results of this study and previously reported data may be due to variation in the JP-8 fuel samples. Fuels differences are due to location of their source and the refinery from which they are produced.

Tables 12 and 13 are summaries of the acute test results with JP-8, JP-8 + 100 (Mobil) additives, and JP-8 + 100 (Betz) additives. Under the conditions of these animal tests, the additive packages did not potentiate the acute effects normally associated with JP-8 jet fuel exposures.

Table 12. Summary of Acute Test Results for JP-8, JP-8 + 100 (Mobil), and JP-8 + 100 (Betz)

Test Material	Skin Irritation	Sensitization	Oral LD_{50} (g/kg)	Dermal LD_{50} (g/kg)
JP-8	Negative	Negative	>5.0	>2.0
JP-8 + 100 (Mobil)	Negative	Negative	>5.0	>2.0
JP-8 + 100 (Betz)	Negative	Negative	>5.0	>2.0

Table 13. Summary of Acute Inhalation Results for JP-8, JP-8 + 100 (Betz), and JP-8 + 100 (Mobil)

Test Material	Inhalation LC_{50} (mg/L)
Vapor	
JP-8	>3.43
JP-8 + 100 (Betz)	>3.52
JP-8 + 100 (Mobil)	>3.57
Vapor + Aerosol	
JP-8	>4.44
JP-8 + 100 (Betz)	>4.39
JP-8 + 100 (Mobil)	>4.54

SECTION 6

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APPENDIX A

DRAIZE[a] SCALE FOR EVALUATION AND SCORING OF SKIN REACTIONS

Parameter	Score
1. ERYTHEMA	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness)	4
2. EDEMA	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raising approx. 1 mm)	3
Severe edema (raising more than 1 mm and extending beyond area of exposure)	4
3. NECROSIS[b]	
No necrosis	0
Slight necrosis (less than one-fourth exposed area)	5
Moderate necrosis (one-fourth to one-half exposed area)	10
Severe necrosis (more than one-half exposed area)	15

[a]Draize, J.H., G. Woodard, and H.O. Calvery. 1944. Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes. *J. Pharm. Exp. Therap.* 32:377-390.

[b]Necrosis, for the purpose of this scoring system, is defined as a chemical denaturation of tissue sufficiently severe to result in fibrotic replacement (scar tissue). Superficial eschar that heals without scar is not classified as necrosis.

APPENDIX B

NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH INTERPRETATION OF SKIN RATINGS[1]

	Rating	Interpretation
Intact skin	0 - 0.9	Non-irritant; probably safe for intact human skin contact
	1 - 1.9	Mild irritant; may be safe for use, but appropriate protective measures are recommended during contact
	2 - 4	Too irritating for human skin contact; avoid contact

[1]Campbell, K. I., E. L. George, L. L. Hale, and J. F. Stara. 1975. Dermal Irritancy of Metal Compounds. *Arch. Environ. Health.* **30**:168-170.

APPENDIX C

GRADING SYSTEM[1] FOR SENSITIZATION TEST

ERYTHEMA	EDEMA
0 - None	0 - None
1 - Very Slight Pink	1 - Very Slight
2 - Slight Pink	2 - Slight
3 - Moderate Red	3 - Moderate
4 - Very Red	4 - Marked

[1]Toxic Hazards Research Unit grading system for sensitization test.

APPENDIX D

SCALE[1] FOR DETERMINING SENSITIZATION POTENTIAL

Sensitization Rate (%)	Grade
10	Weak
20-30	Mild
40-60	Moderate
70-80	Strong
90-100	Extreme

[1]Toxic Hazards Research Unit scale for determining sensitization potential.

APPENDIX E

EXPOSURE ATMOSPHERES FOR JP-8 + 100 (MOBIL): AEROSOL PARTICLE SIZING DATA AND GC FRACTION ANALYSES

The inhalation portion of this study provided an opportunity to quantify the chemical and physical distribution of the complex series of increasingly higher boiling point components of the test material. The chromatography from JP-8 and JP-8 + 100 (Betz) are indistinguishable from that of JP-8 + 100 (Mobil). Both aerosol particle sizing and gas chromatography of the fractions of JP-8 + 100 (Mobil) only were performed. Data were obtained during the course of the JP-8 +100 (Mobil) exposures to define the atmospheres.

Generation

Both the vapor-only and vapor with aerosol exposure atmospheres were generated using two 6-jet Collison nebulizers operated at 62 psi head pressure (1 cfm flow). Two three-neck 250-mL flasks maintained in a 35 °F water bath provided containment, temperature control, and initial impact surface. Fuel was pumped into each flask at a rate below that which caused a buildup (0.5 mL/min). A vertical rise (~5 ft.) from the nebulizer to the chamber entry point along with the filter body acted as an elutriator and aging chamber. Particles too heavy were trapped and puddled in the base of the HEPA filter. At termination of the exposure this drained back into the generator flask. (Source of sample called Drain Back).

Aerosol

Particle size distribution was accomplished using a Lovelace type cascade impactor (seven stages and final filter). A 30-second sample at 20 L/min provided a good impact mass as well as centering of the sample between the fourth and fifth stage. The mass median aerodynamic diameter particle size and Sigma-G were calculated using a program modified for RS/1. One sample was obtained from each vapor with aerosol exposure at approximately the beginning of the third hour of the test. Samples were handled to minimize problems of vaporization from the stages.

Aerosol mass concentration was determined using an in-line extra thick glass fiber filter, 25 mm, (Gelman Sciences, Ind., Ann Arbor, MI) for a 1-L volume sample. A Ram-S in 0-200 μG/mL range was used to monitor the vapor-only exposure, to detect if an aerosol was starting to form. The concentration in the vapor with aerosol exposures was too concentrated for use of the Ram-S. During the vapor only exposures no increase in mass was found on the in-line glass fiber filters in the hydrocarbon analyzer sample line.

The sample for gas chromatography was obtained using a dry midget impinger long enough to collect approximately 0.2 mL.

Vapor

The mass concentration of the hydrocarbon vapor phase was analyzed using Beckman 400 total hydrocarbon analyzers calibrated with hexane standards. The sample (3 L/min) was filtered to prevent line contamination by aerosols.

Chromatography

Three critical samples, original material, aerosol, and vapor, were chromatographed plus one additional, the drain-back fluid. The system used consisted of the Varian 3700, equipped with a 15-M x 0.53-mm SPB-1 column and flame ionization detector. The temperature program was 40 °C to 200 °C at 5 °C/min. Chromatogram display and integration was performed by the Nelson System (Nelson Analytical, Inc., Cupertino, CA). Over 200 peaks were integrated with this system and the normal alkanes acted as markers.

To allow easier quantification and generalization, the chromatograms were divided into slices of summed areas, each ending with and identified by the terminal normal alkane, e.g., C12 slice begins immediately following undecane and is terminated with the area of dodecane. By plotting the percent total area of each slice versus the boiling temperature of its nominal alkane, a generalized picture of the composition is observable and can be compared with the other samples. A plot of the accumulated percentages determines boiling temperature of the approximate 50% point and 16-84% gives a picture of the temperature range composition of the sample.

The differences between the three fuels were not readily observable by the chromatography method in use (See Figure 1). A set of plots of slice data from samples of JP-8 + 100 (Mobil) demonstrate the differences in the component composition of the vapor, aerosol, fuel, as received, and the drain-back samples (Figures E-1 through E-4).

FIGURE E-1. DATA AND PLOTS FROM THE CHROMATOGRAM OF JP-8 + 100 (MOBIL) AS RECEIVED

SLICE CARBON #	BP (C)	MW	CUT OFF TIME (MIN)	AREA	AREA %	CUM AREA %
C8	126	114.2	4.4	461615.3	1.4	1.4
C9	151	128.3	6.92	1713543	5.3	6.7
C10	174	142.3	10.2	5393742	16.6	23.3
C11	196	156.3	13.85	7493122	23.1	46.4
C12	216	170.3	17.5	6125052	18.9	65.3
C13	235	184.4	21.05	5219496	16.1	81.4
C14	254	198.4	24.42	3187787	9.8	91.2
C15	271	212.4	27.65	1884813	5.8	97.0
C16	287	226.5	30.68	639910	2.0	99.0
C17	302	240.5	33.6	237832.5	0.7	99.7
C18	316	254.5	36.4	67692.3	0.2	99.9
C19	330	268.5	40	34919.8	0.1	100.0

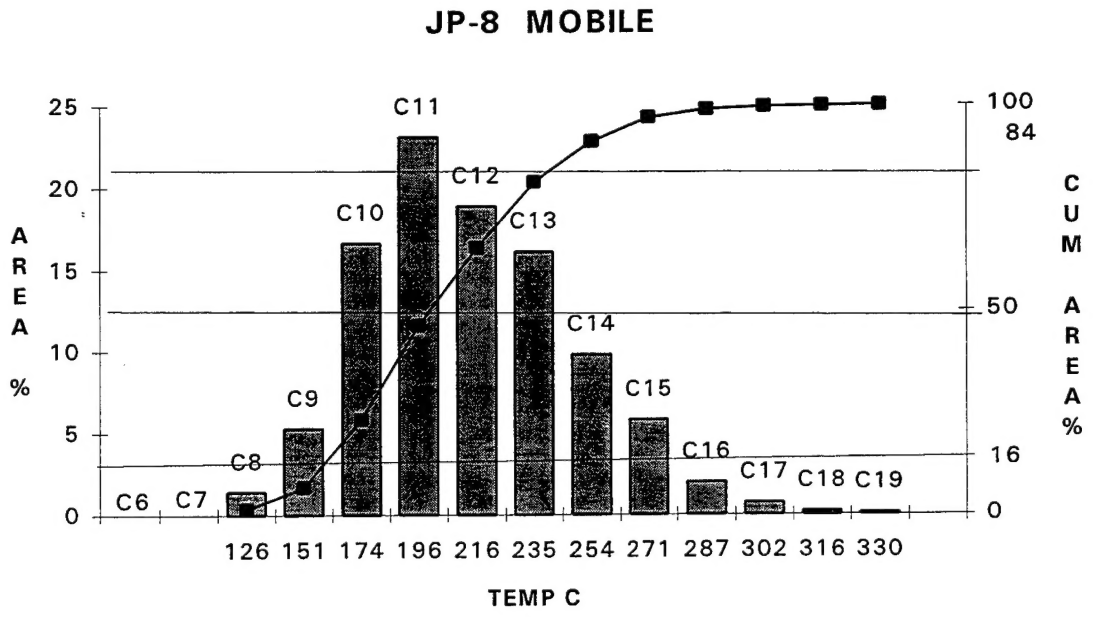

FIGURE E-2. DATA AND PLOTS FROM THE CHROMATOGRAM OF JP-8 + 100 (MOBIL) VAPOR

SLICE CARBON #	BP (C)	MW	CUT OFF TIME (MIN)	AREA	% AREA	% CUM AREA
C8	126	114.2	4.4	6800.6	4.5	4.5
C9	151	128.3	7	23076.5	15.3	19.8
C10	174	142.3	10.3	54675.9	36.2	55.9
C11	196	156.3	13.9	46209.5	30.6	86.5
C12	216	170.3	17.6	12593	8.3	94.8
C13	235	184.4	21.1	4460.3	3.0	97.8
C14	254	198.4	24.5	2690	1.8	99.6
C15	271	212.4	27.7	641.3	0.4	100.0

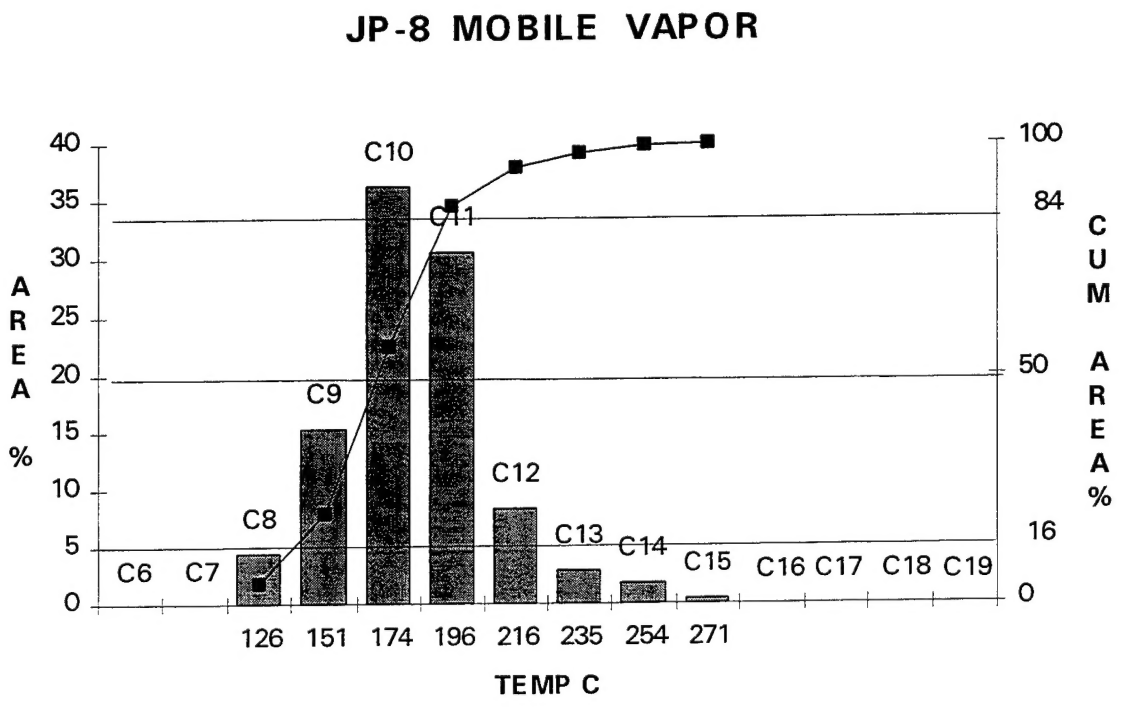

FIGURE E-3. DATA AND PLOTS FROM THE CHROMATOGRAM OF AEROSOL JP-8 + 100 (MOBIL)

SLICE CARBON #	BP (C)	MW	CUT OFF TIME (MIN)	AREA	AREA %	CUM AREA %
C8	126	114.2	4.4 0	0.0	0.0	
C9	151	128.3	7 5649	0.1	0.1	
C10	174	142.3	10.3 100579.7	1.5	1.6	
C11	196	156.3	13.9 360402	5.3	6.8	
C12	216	170.3	17.6 747226.1	10.9	17.8	
C13	235	184.4	21.1 1621108	23.7	41.5	
C14	254	198.4	24.5 1806179	26.4	67.9	
C15	271	212.4	27.7 1420049	20.8	88.7	
C16	287	226.5	30.7 577556	8.5	97.1	
C17	302	240.5	33.6 195398.5	2.9	100.0	

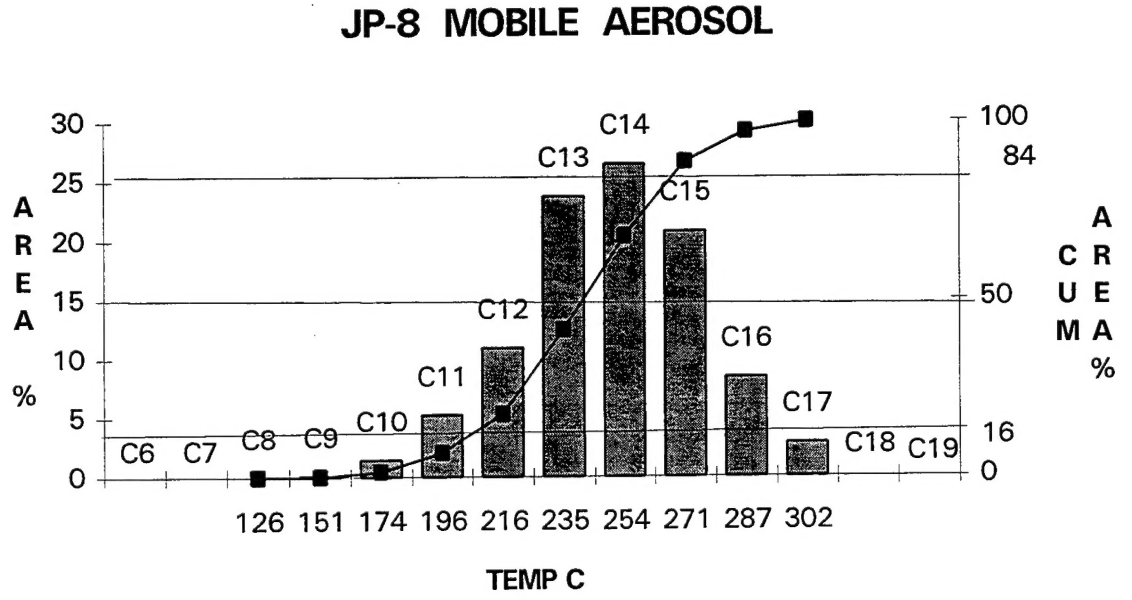

FIGURE E-4. DATA AND PLOTS FROM THE CHROMATOGRAM OF SPENT MATERIAL FROM AEROSOL EXPOSURE OF JP-8 + 100 (MOBIL)

SLICE CARBON #	BP (C)	MW	CUT OFF TIME (MIN)	AREA	AREA %	CUM AREA %
C8	126	114.2	4.4	11757.1	0.1	0.1
C9	151	128.3	7	152689.1	1.1	1.1
C10	174	142.3	10.3	1279107	8.8	10.0
C11	196	156.3	13.9	2866337	19.8	29.7
C12	216	170.3	17.6	2983906	20.6	50.3
C13	235	184.4	21.1	3092551	21.3	71.7
C14	254	198.4	24.5	2302262	15.9	87.6
C15	271	212.4	27.7	1226002	8.5	96.0
C16	287	226.5	30.7	395027.6	2.7	98.7
C17	302	240.5	33.6	142701.4	1.0	99.7